PP20

TOWARDS STANDARDIZATION OF OA CARTILAGE HISTOPATHOLOGY; REPRODUCIBILITY OF PROPOSED SYSTEM (THE BARCELONA DRAFT)

Hyllested. J.L., Veje, K, Ostergaard, K.
Osteoarthritis Research Unit, Institute for Inflammation, National University Hospital, Copenhagen, Denmark.

Objective: The Histologic/Histochemical Grading System developed by Mankin and coworkers is neither adequately reproducible nor sufficiently valid. At the OARSI congress 2000 in Barcelona, Spain a new Histologic / Histochemical Grading System was proposed. The objective of this study is to evaluate the intra- and interobserver reproducibility of the proposed system.

Methods: Human articular cartilage was obtained from macroscopically normal and OA (Collins Mc-Elligot grade 0 and III/IV, respectively) knee joints. Sections of central and peripheral regions of normal samples and sections of OA samples containing severe, moderate and mild OA changes were produced. A total of 88 sections were graded twice by two observers. The sections were graded and staged, and a score calculated by multiplying the grade with weighted stage.

Results: Intra- and interobserver reproducibilities

Observer	Exact reproducibility	Reproducibility within 8 patients	Average difference	Range	Median	95 % CI
A	49%	84%	2.6	(-31)-(36)	15	6-18
B	49%	92%	-1.4	(-24) - (36)	9.5	5-17
A vs. B	48%	80%	2.0	(-40) - (34)	12	8-20

Average difference: Average difference between first and second score for each observer, and between the first score of both observer.
Range: Range of differences between first and second score for each observer and between first score of both observers.
Median: Median of the first and second score for each observer and for the first score of both observers.
95% Confidence Interval: The range of scores which contains the true median with probability 0.95.

Conclusion: The proposed system is reproducible and could form the basis of a new histologic/histochemical grading system.

PP21

INDUCTION OF APOPTOSIS IN HUMAN CHONDROCYTES BY HYDROSTATIC PRESSURE

C.J. Malemud, T.M. Haqqi, K. J. Jepsen,
N. Islam, M. Kraay, J. Welter, V.M. Goldberg
Case Western Reserve University, Cleveland, Ohio, USA

Aim: The aim of this study was to determine whether hydrostatic pressure (HP) induced apoptosis in human chondrocyte cultures derived from osteoarthritic (OA) cartilage.

Methods: Human chondrocytes were enzymatically dissociated from the "resident" cartilage of OA femoral heads. Chondrocytes were subjected to HP (0.1-5 MPa) for 2 hrs. or 4 hrs. in a device specifically constructed for these studies. Characteristic markers of apoptosis induction were assessed by SDS-PAGE, RT-PCR and Western blotting.

Results: HP resulted in DNA fragmentation consistent with induction of apoptosis. Cleavage of poly-ADP-ribose polymerase (PARP) was detected by SDSPAGE. HP induced *c-myc, p.53* and *Bax-aipha* gene expression and suppressed *bcl-2* expression. Several other genes, including *c-fos, ICAM-3* and *p21/waf* were unaffected by HP. Non-loaded chondrocytes did not synthesize c-myc or p53 protein on Western blots. HP induced c-myc and p53 protein after 4 hrs. of HP at 5 MPa. Bcl-2 protein synthesis was suppressed as a function of loading time and level of HP. By 4 hrs. at 5 MPa, bcl-2 protein was undetectable by Western blotting.

Conclusions: The induction stimulus resulting in apoptosis in human OA cartilage remains unknown. The present results showed that HP-loading of human OA chondrocytes at levels consistent with those measured during normal gait induced apoptosis *in vitro*. The mechanism is consistent with induction of pro-apoptotic gene expression (i.e. *c-inyc, p.53, Bar-alpha)* and suppression of anti-apoptotic gene expression (i.e. *bcl-2).* HP also resulted in PARP cleavage consistent with activation of caspases. Indeed, HP also increased caspase-3 gene expression in human OA chondrocytes.

PP22

NITRIC OXIDE INHIBITION OF CARTILAGE RESPONSE TO GROWTH FACTORS

K Studer, K Decker, H Georgescu
VA Med Center and University of Pittsburgh School of Medicine, Orthopaedic Surgery, MSRC, Pittsburgh, PA, USA

Aim: The aim of this study was to determine the mechanisms involved in the insensitivity of arthritic cartilage to anabolic actions of IGF-1 and other growth factors.

Methods: Cartilage was obtained secondary to total knee arthroplasty for degenerative joint disease and 15-25 mg slices or isolated chondrocytes (AC) maintained in tissue culture. In some studies, AC from normal human talus or rabbit articular cartilage were used. Responsiveness was measured as increases in proteoglycan synthesis and stimulation of chondrocyte proliferation.

Results: Arthritic human cartilage showed minimal response to IGF-1 or 5% fetal calf serum (FCS) (increases of 24 and 36 pmol/l0 mg wet wt respectively). LNMA inhibition of NO synthesis doubled the increases to 52 and 69 pmol/10 mg. KT5823 is a specific inhibitor of cGMP kinases; when it was added to the cultures before IGF-1 or FCS, proteoglycan synthesis was similarly increased, thus implicating NO dependent increases in cGMP dependent kinase actions in arthritic cartilage insensitivity to both IGF-1 and the anabolic factors contained in serum. When normal human talus chondrocytes were exposed to NO from 0.01 mM SNAP, basal proliferation, as measured by ^{3}H-thymidine incorporation, decreased (from 943± 120 to 572± 100 dpm)as did that in response to 5% FCS (from 8763±6 19 to 4640±686 dpm) and 5 ng/ml bFGF (from 5043±652 to 654±77 dpm, a value not different from basal). AC transduced with iNOS produced conditioned media concentrations of nitrite similar to those seen in the presence of 0.01 mM SNAP. In these cells proliferation in response to bFGF was inhibited, however it was partially restored by 50 uM of the cGMP kinase inhibitor Rp-8-pCPT-cGMPs. 100 uM of the cGMP analog 8-pCPT-cGMP inhibited the proliferation in response to bFGF and IGF-1 by 50% and 40 %.

Conclusions: Arthritic cartilage IGF-1 and FCS stimulated proteoglycan synthesis is enhanced by the inhibition of NO synthesis or cGMP kinase action. IGF-1, bFGF, and FCS stimulated proliferation are also inhibited by NO; these effects are mimicked by exogenous cGMP and are partially reversed by inhibitors of cGMP dependent kinase. The data supports an important role for NO in arthritic cartilage insensitivity to growth factor stimulation and identifies cGMP dependent mechanisms as one of the effectors.